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Review Article

Deficiency of Vitamin D_1: The Principle of Carcinogenesis

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Abstract

Vitamin D_1 (hexahydroxy, 5-β, 6-keto,7-dehydrocholesterol; 20-hydroxyecdysone) was accidentally discovered in the search for an insect moulting hormone in 1965. Recent studies [5] provide evidence, however, that the true biological nature of this partly water-soluble derivative of cholesterol is not an animal hormone. It is a long neglected, biologically active vitamin D_1 which does not need to be activated in the skin by UV-light. Unlike other purely lipid soluble cholesterol derivatives, vitamin D_1 is widely distributed in yeast, mushrooms and lower and higher plants. Animal tissues do not seem to synthesize vitamin D_1 *de novo*; they obtain it from vegetable food, herbivores and possibly also from intestinal symbiotic flora. The availability of vitamin D_1 in human blood may be essential for the construction of cytoplasmic membranes in dividing cells during somatic growth and regeneration. In contrast to the common provitamins D_2 and D_3, vitamin D_1 is biologically active without UV-light in all organisms. In the human body, vitamin D_1 causes a plethora of beneficial health effects: tonic and antifatigue, anabolic growth of the bones and flesh in juveniles, increased muscle performance or increased immunity in newborn babies. Recently, there have been reports about anti-Alzheimer, anti-carcinogenic and anti-muscular degeneration properties. Here it has been suggested that vitamin D_1 prevents the formation of malignant, polynucleated tumors.

Keywords: *Vitamin D_1; Carcinogenesis*

The story of antirachitic vitamin D

With only a few exceptions, the growth of all multicellular organisms proceeds because of increased cell numbers, not cell size. Growth of the body and regeneration of the diploid cells proceeds in the form of mitotic cell divisions. In the 19th century, malnutrition and especially a deficiency of milk, produced serious growth retardation in children, known as Rickets disease (*rachitis*). The syndrome was traced to a deficiency of vitamin D. The pioneers investigating Rickets disease 100 years ago noticed the association of the disease with 7-dehydrocholesterol. At that time, Isoprenoid chemists believed that cholesterol and its derivatives were nonpolar, lipid soluble animal sterols (zoosterols) that were absent in plants. For this reason, they searched for vitamin D only in animal sources, like muscles, liver or kidneys. In 1930, a steroid chemist Adolf Windaus and his colleagues isolated two secosterol compounds related to 7-dehydrocholesterol from pork skin. They became generally known as provitamins D_2 (ergocalciferol) and D_3 (cholecalciferol). The provitamins D_2 and D_3 were biologically inactive. Their biological activation was tentatively identified by exposure of animal skin to UV-light. Provitamins D_2 and D_3 have been used as the regular form of vitamin D since 1930 [1,2]. The characteristic features of D_2 and D_3 are their exclusive lipid solubility, biosynthesis in animal kidneys, livers or fish oils and virtual absence in plants [2].

The biological activation of D_2 and D_3 by UV-light represents a real difficulty. For example, the skin of most animals is protected from UV-radiation by a dense hair cover. Moreover, the purely lipid soluble compounds, like free sterols, waxes or D_2 and D_3, with just one polar hydroxylic group against 27 olefinic, nonpolar CH_2 methylene units characterize a metabolically inefficient compound, similar to inert waxes [5]. In contrast, the biologically effective derivatives of 7-dehydrocholesterol, such as the cholecalciferol triol (rocaltrol), containing 3 hydroxylic groups and, especially, the polyhydroxylated 20-hydrocyecdysone (vitamin D_1) with 6 or 7 hydroxylic groups are easily metabolized [5] (see below). I can reasonably conclude that the free cholesterol present in human blood could actually be a dehydroxylation metabolic end product of the vitamin D_1, which enters the body through a vegetable diet.

The story of vitamin D_1

It appeared that the biological activity of samples tested for vitamin D increased during the vegetation season. In addition, UV-irradiation of the samples also enhanced the investigated biological activity of vitamin D [2,3]. In the chemical laboratory, UV-light was used for the conversion of cholesterol into 7-dehydrocholesterol, the expected structural component of vitamin D. In order to increase biological activity, the research group of A. Windaus irradiated the provitamin D_3 by UV and obtained a mixture of compounds which exhibited the biological activity of vitamin D without further dependence on UV-light [3]. They vigorously tried to identify the active ingredient, named vitamin D_1. In spite of numerous efforts, the identification of the vitamin completely failed [3]. The structure of the mysterious vitamin D_1 remained unknown [4,5].

In retrospect, I think that A. Windaus and his colleagues [3] probably induced an undesired hydroxylation of 7-dehydrocholesterol. At that time, they believed that all derivatives of cholesterol should be nonpolar and purely lipid soluble. The polar sterol fractions were most probably discarded without being tested for vitamin D [5]. Due to this eventuality, the vitamin D_1 story of Windaus [3] has never been elucidated. The effect of hydroxylation on the biological activity of vitamin D is supported by cholecalciferol triol (1,3,25-trihydroxy cholecalciferol) [20].

Partly water soluble 7-dehydrocholesterol (Ecdysone)

In 1965, an outstanding German biochemist P. Karlson and his co-workers accidentally discovered a new group of polyhydroxylated 5-β.6-keto, 7-dehydrocholesterol in the search for an insect moulting hormone. They extracted 500 kg of silkworm pupae and obtained 25 mg of crystalline material named ecdysone [6]. The solubility of the newly discovered compound with 6 polar hydroxylic groups was slightly water soluble in contrast to all other, exclusively lipid soluble sterols. Originally, ecdysone was described as a hormone of the insect prothoracic gland. Surprisingly, soon after the crystallographic identification of the ecdysone structure, phytochemists from different countries reported the presence of this insect hormone in various species of plants [7,8]. In 1974, a comprehensive review of insect hormones [7] listed dozens of phytochemical studies on the widespread occurrence of the compounds in plants, including changes in the displacement of hydroxylic groups across the sterolic molecule. The most common structure was 20-hydroxyecdysone, generally known as ecdysterone and the group of these compounds received a generic term ecdysteroids (ECD).

With increasing data, it became obvious that these slightly polar, polyhydroxylated derivatives of 7-dehydrocholesterol were a regular secondary substances of plants [13,15]. For a long time, nobody could explain what the physiological role of an insect hormone in a plant system would be. Curiously enough, the “hormones” occasionally accumulate in plants in unbelievably large quantities, exceeding a million-fold their usual content in animals [7,9,10,13]. We have analyzed a possibility that ECD could eventually function as phytohormones. However, extensive assays using several phytohormones revealed no hormonal mode of action of ECD in plants [12]. In 1979, a controversial but ultimately accurate explanation proposed that ECD were the reserve materials supporting the growth and proliferation of plant tissue and cells [13-16].

The biological status of ECD

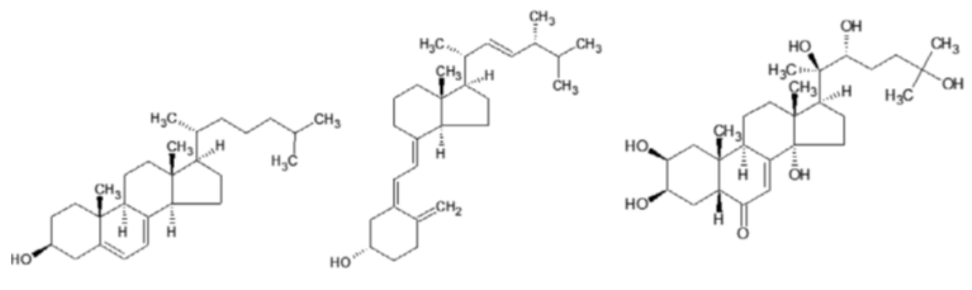
The nature of ECD has been critically discussed for many years [14,16]. More recent studies on the biological nature of these materials that are present in plants, insects, vertebrate animals and humans, suggests that this type of natural substance should deserve to have a more important biological significance than being just a hormone of the tiny prothoracic gland of insects [5,8,13-17]. In plants, ECD are produced in the green leaves and needles during the vegetative growth season. From the leaves, they get translocated into reserve organs, usually seeds or winter roots. In the next season, they are incorporated into the structures of the new growing system. Occasionally, they are stored in the roots or seeds in enormous quantities (over 3% of dry root) [11]. For example, the Nordic Siberian plant, *Leuzea carthamoides Iljin*, contains 2.5% of ECD in dry seeds [7,9,10,13]. Moreover, the dry tropical plant, *Cyanotis arachnoides,* from Somalia, Africa, may contain up to 10% of this (tentatively insect hormone). Their products are sold to sports athletes as a doping for enhanced muscular performance (Alibaba Co., China).

The presence of ECD in plants has been frequently studied and publicized [7,9,10,23-29]. In spite of such overwhelming publicity, the articles from phytochemists have notoriously adhered to the old concepts of an insect hormone [9,10,24,26,39]. Some of these articles have made distinction between phytoecdysteroids and zooecdysteroids [10], without respect for whether a compound was produced in an animal or just came into the body with vegetable food. The new term "dietary phytoecdysteroids" [23] has been used for ECD previously found in plants, like spinach, *Chinoa,* corn and several other vegetables [23]. There was no mention about the possible vitaminic role of ECD [14-18]. The presence of ECD in plants was often explained by an old and abandoned theory suggesting the deterrent action against insect herbivore pests [7,37,38]. In human athletes, ECD appeared as absolutely nontoxic natural drugs [25-27]. Extremely large dosages were rapidly excreted in urine within several minutes [23]. In insects, there exists a phenomenon named hyperecdysonism by Williams in 1968 [36]. The increased amounts of ECD are very vulnerable to the feeding insect larvae. They cause adverse hypervitaminic effects connected with complete arrest of feeding, and locomotion [15]. It is also associated with muscular paralysis, decrease of total metabolism. In normal development, this type of physiological syndrome is characteristic for preparation of the larval stage for moult [5].

A vitamin or hormone?

The late Prof. H. E. Hinton of Bristol University insisted that the terms of science should correspond with their facts in nature. The definition of a vitamin is considerably different from that of a hormone. Vitamins are secondary compounds of plants, which are essential for various physiological functions in animals who do not produce them. Hormones are secreted from specialized animal endocrine glands and act only on the determined target tissues containing the respective nuclear receptors. Hormones act at incredibly small concentrations (10^{-9} M) and never accumulate. I have studied insect hormones for several decades [5,7,12-14,16,19,27]. It was possible to extirpate prothoracic glands from a large number of insect larvae [19]. In contrast to the initial conclusions on the origin of ecdysone [6], it was found that the presence or absence of these glands did not affect the larval moults [19]. In addition, the endogenous peaks in the concentration of ECD in insect haemolymph [35] have also been found to originate in pupal stages with previously disintegrated prothoracic glands [5,18]. Evidently, ecdysone was not the hormone of the prothoracic gland. During the prepupal and pupal peaks of ECD that is present in nonfeeding insects, many disintegrating tissue and organs are sequestering ECD into haemolymph, which is also the case of the disintegrating prothoracic gland [18,19]. The functions of ECD both in plants and animals does not fit the definition of a hormone [14-17]. The available experimental data indicate that ECD are primarily the products of the plant kingdom [7,9,10,13-16].

I found that ECD did not induce insect ecdysis at all [5,14,16,18,19,26]. Due to their essential role in the mitotic cell divisions in all organisms [5], I proposed to call these natural compounds by the generic name vitamin $D_{1,}$ which was originally suggested by Windaus in 1930 [5,7,12-14,16-18,22] (See figure 1). Finally, there remains the most disturbing terminological problem of ECD with the suffix "sterone". This suffix is reserved in endocrinology for the real steroid hormones (e.g. corticosterone, testosterone, progesterone). Since ecdysone and other ECD are not hormones, I strongly recommend that these natural substances will be correctly termed as the secondary substances of plants, ecdysteroids, vitamin D_1 [5]. Perhaps the best identification of ecdysteroid compounds will be the structural relation with the original compound, ecdysone (e.g. 20-hydroxyecdysone, 5, 20-dihydroxyecdysone, etc).



A
(3b)-cholesta-5,7-dien-3-ol;
7-dehydrocholesterol

B
(3*S*,5*Z*,7*E*,22*E*)-9,10-secoergosta-5,7,10,22-tetraen-3-ol; calciferol

C
(2b,3b,5b,22*R*)-2,3,14,20,22,25-hexahydroxycholest-7-en-6-one;
20-hydroxyecdysone, vitamin D_1

Figure 1. *Chemical structures of 7-dehydrocholesterol (A.), Vitamin D3 (cholecalciferol, (B.) and vitamin D1 (20-hydroxyecdysone).*

A brief recapitulation of the effects of vitamin D_1

According to the available data, vitamin D_1 is an essential building material in the formation of cytoplasmic membranes during mitotic cell divisions, both in the animal and plant kingdoms. It is a natural drug with beneficial health effects that is widely contained in plants [12-17]. There are several reasons why such an important vitamin remained unknown for a long time: 1. The first reason is that the textbooks of isoprenoid chemistry stated that cholesterol and its derivatives were strictly nonpolar, water insoluble compounds that were not present in plants; 2. Another reason may depend on the notorious belief that ecdysone was an insect moulting hormone; 3. The third reason is the amphoteric, partly lipid and partly water solubility of the polyhydroxylated derivatives of 7-dehydrocholesterol; 4. A further reason may be in the practical disappearance of the vitamin from the pool of extractable materials after its incorporation into the cytoplasmic structures of the mitotically divided plant or animal cells; 5. I am also convinced that the reason for overlooking the role of vitamin D_1 is the confusing belief in the necessity of UV-radiation for activation of the provitamins D_2 and D_3 and; 6. Orientation of research projects into the study of hormonal nuclear receptors [39].

Plants

Perennial plants produce vitamin D_1 in green leaves during vegetation. The vitamin is incorporated into the leaf structure and further used for growth and tissue proliferation. It is transported and stored in roots, seeds and epidermal buds for development in spring [5,7,24-27]. Certain plants can store large amounts of vitamin D_1. Among the most common, we can find, for example, *Leuzea carthamoides, Cyanotis arachnoidea, Ajuga turkestanica or Serratula coronata*, that are mostly used as rejuvenating drugs [24-27,39]. The occurrence of vitamin D_1 in plants was reported almost immediately with Karlson's disclosure of the structure of ecdysone [6,8,11]. It has been positively established that vitamin D_1 does not act in plants as a phytohormone [21]. Most reports on "insect hormones in plants" (ECD) describe a different position of the hydroxylic groups [7-9,16,23,24,26,27]. The compounds containing at least 3 hydroxylic groups are biologically active in insects [5].

Derivatives of vitamin D_1 with 7 hydroxylic groups (5,20-dihydroxyecdysone) were found in the rhizomes of a fern (*Polypodium vulgare*) [11], suggesting that vitamin D_1 occurs also in lower taxonomic groups of plants. In addition, the biosynthesis of vitamin D_1 has been also found in primitive plants like yeasts and mushrooms [29]. We may thus assume that the ability to synthesize vitamin D_1 is a common evolutionary adaptation across the whole multicellular plant kingdom.

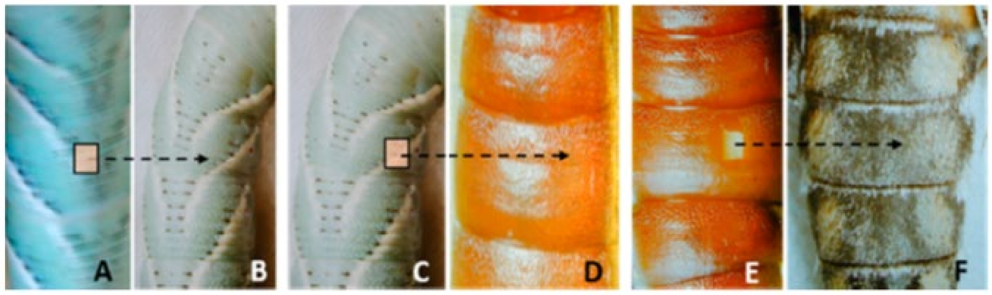


Figure 2. *Homochronic (similar) regeneration of epidermal excisions during development of Manduca sexta L. The excised epidermal window (3 x 3 mm) always regenerated into the homologous epidermal structures (indicated by an arrow). A–penultimate larval instar, B+C- last larval instar, D+E- pupal instar, F-adult stage (from (5)).*

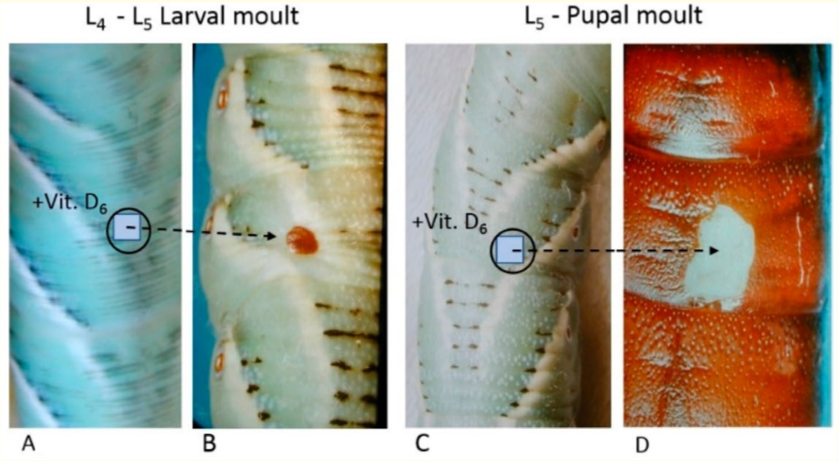


Figure 3. *The heterochronic (structurally different) regeneration of excised epidermal area of 3 x 3 mm in presence of vitamin D1 (local application in melted gelatine; D6 is an older term for vitamin D1) in larvae of Manduca sexta L. Mitotic divisions induced by epidermal injury (in absence of juvenile hormone and in the presence of vitamin D1) resulted in premature development of the future, pupal stage. After the next moult, this became evident as a patch of heterochronic pupal cuticle on the larval body (B), or a patch of incomplete adult cuticle on the pupal body (D).*

Figure 4. *The advanced hypervitaminic, pupal-adult intermediates (adultoids) of Manduca sexta L. The mixture of pupal and adult cuticles was achieved by injection of 5 µg of vitamin D1 (20-hydroxyecdysone) into 5-day-old pupae.*

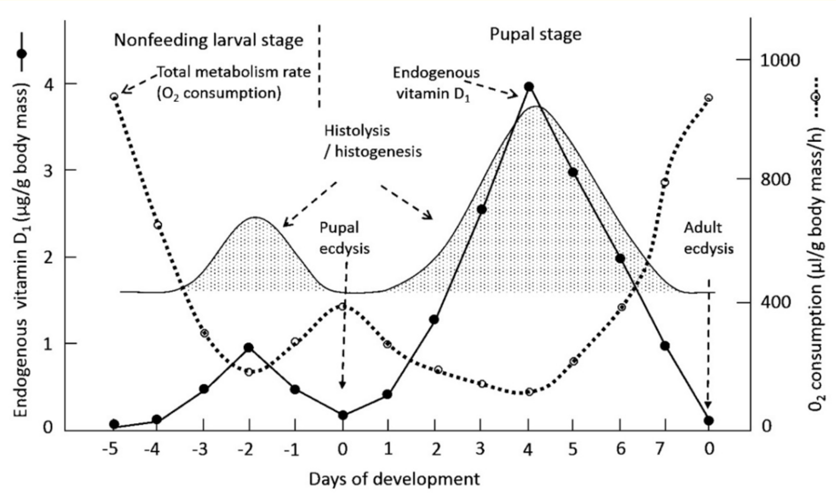


Figure 5. *Relationships between endogenous peaks of vitamin D1 (full line), total body metabolism (O2 consumption; dotted line) and the morphogenetic process of histolysis/histogenesis (dotted area) during metamorphosis of the wax moth, Galleria mellonella L. Note that the maximum concentration of vitamin D1 in the body occurs at the time of minimum metabolism (adapted from (5)).*

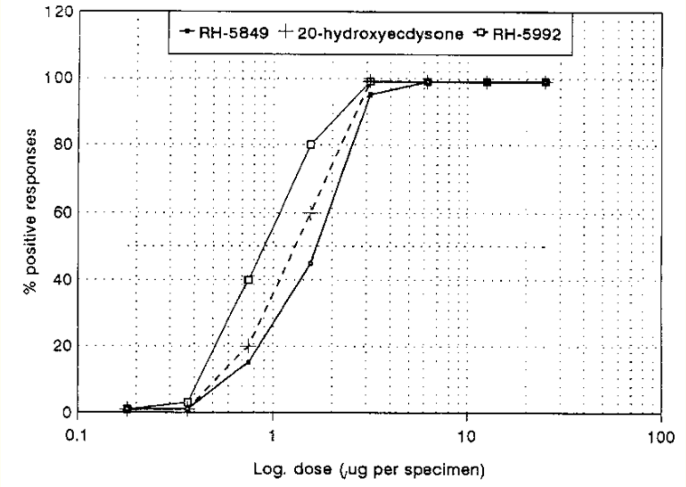


Figure 6. *Standardized biological assay of vitamin D1 (20-hydroxyecdysone) in ligatured larvae of the wax moth, Galleria mellonella L. Positive effect was determined by the release of new, pupal cuticle (15). The effective dose ID-50 was 1.2 µg/150mg larva. The bisphenylhydrazine biological ecdysteroid mimics RH-5849 and RH-5992s show similar dose-response relationships (adapted from ((15/ 43/44 (,47)).*

There appears to be a question of whether animals can also *de novo* synthetize vitamin D from acetate, or whether they obtain it only from plants. The provitamins D$_2$ and D$_3$ are thought to be produced in animal liver or kidneys and vitamin D used to be indicated as a hormone [1,2,39,52,58]. The problem was not experimentally investigated because cholesterol was not considered to be a plant sterol and nobody expected that a cholesterol derivative can enter the body through vegetable food. Our data about the almost ubiquitous occurrence of vitamin D$_1$ across the plant kingdom allowed me to speculate that, similarly to the case of all other vitamins, animals can get vitamin D$_1$ exclusively from plants. Animals originally evolved as herbivores. They received vitamin D$_1$ from plants. Predators could get the vitamin from the herbivores. According to this idea, animals learned across millions of years of animal/plant coevolution to avoid the burden of complicated triterpenoid biosynthesis, received the vitamin D from food and saved the space of intracellular organelles for specifically animal structures [5,21].

It is not certain whether the ecdysone found by Karlson [6] was produced in the silkworm or entered the body with plant food. As a matter of fact, ecdysone, which differs from most other ECD by the lack of 20-hydroxyl group, is present also in the mulberry food plant of the silkworm [9,10,17]. In *Leuzea carthamoides,* we can find the largest accumulation of vitamin D$_1$ (20-hydroxyecdysone) content in the early spring sprouts (4,5% of dry material), a considerably smaller amount (0.5%) in growing green leaves, and very little (0.01%) in autumn leaves. This shows that the vitamin incorporated into the growing structures of the plant system cannot be extracted. The example of *Leuzea* points out that the roots and other reserve organs of many common plants of Europe can be a good source of vitamin D$_{1,}$ however, this has never been investigated due to the belief in insect hormone.

In comparison with terrestrial organisms, our knowledge concerning the presence of vitamin D$_1$ in marine plants and animals is still rather incomplete. The presence of ECD has been well documented in marine crustacea (crustecdysone) [7,36]. Relatively large concentrations of ECD were found in marine invertebrates, pycnogonids [54]. In an analogy with terrestrial food chains, it can be assumed that vitamin D$_1$ will also be produced in algae and other marine flora and will be also used as an important growth factor during the mitotic divisions of cells. I have no data about the possible production of vitamin D$_1$ in fish, as the most common marine animal (although the recommended sources of vitamins D_2 and D_3 are from fish oils). A possibility of additional synthesis of vitamin D_1 by intestinal symbiotic flora in fish should be also considered. The recent article about distribution of ECD in plants and their role in human health [39] explains the reasons for the existence of these compounds by using the old theory of the deterrent action of insect hormones against insect pests. The theory was criticized many times [37,38], until it was experimentally established that phytophagous animals develop resistance [40,41].

Insects

The story of ecdysone started by its crystallographic identification in silkworm pupae by Karlson and his colleagues [6]. In 1965, a small sample of the compound was sent to a respected authority in insect hormones, Prof. C. M. Williams of Harvard University, who investigated insect metamorphosis hormones [37]. I used to be a co-worker of Prof. Williams at that time. We found that ecdysone stimulated development in decerebrated silkworm pupae, the effect which was at that time ascribed to insect prothoracic glands [37]. The result was communicated to Prof. Karlson, who automatically combined ecdysone with the hormone of prothoracic glands [6]. Almost immediately after the public disclosure of the chemical structure of ecdysone, similar compounds were found in plants by Nakanishi and his colleagues [7,8] and by numerous phytochemists [9,10,24-27,36]. I have investigated insect hormones for a long time [42] and offer here a brief account for the most important findings on ecdysone (vitamin D_1) and other ECD, as follows: 1. Feeding larvae and diapausing stages show minimum concentrations of vitamin D_1 in the body [5,16]; excessive amounts received from food are excreted; 2. Exogenous injections of D_1 (1 - 10 µg) in the feeding larvae are also rapidly inactivated by excretion [5,40]; 3. Large dosages (over 100 µg) injected into feeding larvae cause a syndrome of hypervitaminosis, characterized by immobility, caused by paralysis of all somatic muscles, followed by a decrease of total body metabolism [5,15]; 4. The hypervitaminic syndrome occurs without cell divisions and results in premature, precocious secretion of new cuticle [15]; 5. Artificially induced cell divisions by injury in the presence of vitamin D_1 caused the premature formation of epidermis of future developmental stage (patches of pupal cuticle on larval body, (for more data see figure 2 and 3), the consequence of hypervitaminosis in pupae of *Manduca sexta* are shown by pupa-adult intermediates in figure 4; 6. The nonfeeding metamorphosis stages of insects exhibit special endogenous peaks in the content of vitamin D_1 in haemolymph. The peaks are associated with histolysis/histogenesis reorganisation of tissues during the metamorphosis process [5] (For further information see figure 5); 7. In addition to chemical analysis, the content of vitamin D_1 can be determined by biological assay using ligatured larvae of the waxmoth (*Galleria mellonella*) with the arrested development (ED-50 dose is 1.2 µg/larva) [15,19,28,43,44] (See figure 6); 8. There exist bisacylhydrazine ecdysteroid mimics (RH-5849 and RH-5992 and others) which stimulate formation of new cuticle in insects (Figure 4) [44]; So far, it is not known whether these ecdysone agonists could also mimic the action of vitamin D_1 in vertebrate animals [43,44]. Unlike mammals, where the process of postembryonic morphogenesis develops concealed inside the uterus, the postembryonic development of insects occurs in freely accessible larval, pupal and adult stages. It can be investigated easier and the results are described in the continuation of the above vitamin $D_{1,}$ as follows: 9. During the postembryonic morphogenesis, the individual diploid cells divide exactly according to the Mendelian rule, all-or-nothing, established for early postzygotic development [45]; 10. Endocrinological investigations revealed that ecdysone (vitamin D_1) was not an insect hormone and the prothoracic glands released ecdysone only during their programmed disintegration during the short prepupal period [18,19]; 11. The selective developmental advantage of this mechanism depends on the fact that the outlived, disintegrating larval somatic tissues enable reutilization of the vitamin D_1 to the newly growing, proliferating tissues of the next, pupal or adult stages [18]; 12. The endogenous peaks of vitamin D_1 occurring mostly in the nonfeeding metamorphosis stages are formed also during the embryonic period, before the existence of prothoracic glands [46]; 13. The endogenous peaks of vitamin D_1 cannot be produced by prothoracic glands because they are present also in specimens with completely extirpated neuroendocrine systems [48]; 14. The recent phytochemical literature claims that the *raison d´etre* of ECD depends on resistance of plants against herbivores [39], however, this is a very old hypothesis that was first published in 1979 [13], long after the elucidation of the homeostatic function of ecdysone in insect development [47] and before elucidation of the vitaminic nature of ecdysone [5,12,14,16,35]; 15. The biological activity of vitamin D_1 does not depend only on 5 or 7 hydroxylic groups [7], but also on other specific structural features (turkesterone with C-11 hydroxyl is most active on Dipteran insects, *versus* cyasterone with a different side chain is most active on Lepidopterans) [28]; 17. Other important structural features are the 5of A/B sterolic rings and the conjugated 6-keto,7-dehydro unsaturation resulting in strong electron-accepting property of the compound [7,36]; 18. The most important physiological feature of vitamin D_1 in insects is the direct correlation between the size of the endogenous ECD peaks and the extent of larval/pupal transformation [5,32,33]; 19. On the other hand, there exists inverse proportionality between the extent of tissue proliferation and the total body metabolism [50]; 20. Physiological relationships between vitamin D_1 and insect metabolism, described under 18 and 19, confirm that the biological functions of the vitamin may be the same in all organisms, including plants, animals and humans [5,7,39].

Vertebrates

After the discovery of growth stimulating effects in insects, anabolic effects were found in mice [7,36]. Historically, the first systematic investigation of ECD effects in vertebrates were initiated in the former Soviet Union. An interesting story related to this topic was connected with a research stage of N.K. Abubakirov, head of the Soviet All-Union Institute of Natural Compounds in Tashkent (Uzbekistan) and Czechoslovakian Academy of Sciences in Prague. At that time, the team of Prof. F. šorm isolated 5,20-dihydroxyecdysone from the fern *Polypodium vulgare* [11]. The story continues with the discovery that a Siberian plant, *Leuzea carthamoides* Iljin, substantially increased the production of meat and milk in cattle. A sample sent to Prof. Abubakirov for analysis was easily identified as 20-hydroxyecdysone [23]. From that time, the Soviets launched a wide search for these compounds (ECD) in other plants. They published a number of papers in the local journals, mostly in the Russian or Ukrainian languages [7,17]. Unfortunately, these data were mostly ignored, the main topic was pure phytochemistry and ecdysteroid receptors [7,24,30,36,39,51,52]. A review of these old Russian articles [17] revealed that ECD were natural vitamins, widely distributed in plants, with a plethora of beneficial health effects in humans.

The frequently reported effects of 20-hydroxyedcdysone (vitamin D_1) in vertebrates were the dietary anabolic growth effects in juveniles [5,7,17,24,39,51,52]. The daily peritoneal injections of vitamin D_1 in mice [55] caused anabolic growth effects in female juveniles but not in the male juvenile mice. In adults, the anabolic effects appeared in both sexes. It was concluded that 20-hydroxyecdysone had the character of an essential vitamin [55]. Dietary effects of vitamin D_1 in Japanese quails (*Coturnix japonica*) [22,50] confirmed the most frequently reported anabolic effects on growth during the whole life cycle. Practically similar anabolic effects were found in similar feeding experiments with chicken broilers. In contrast, the dietary addition of vitamins D_2 (ergocalciferol) and D_3 (cholecalciferol) were completely ineffective [30]. The endogenous concentration of vitamin D_1 in the blood of Japanese quails was relatively low. Dietary addition of vitamin D_1 into a standard diet resulted in a dose-dependent increase of the vitamin and its storage at the corresponding level in the blood [22]. The storage of vitamin D_1 in animal blood is a physiologically very important phenomenon. That animals homeostatically store the vitamin in their blood, eliminating excess or deficiency. Excessive amounts are excreted, similarly to the case in insects [5] and in the human body [25].

There are a number of vertebrate animals who live as predators. They cannot receive vitamin D_1 directly from plants. Do they get enough vitamin from the blood of their herbivore prey? This problem has never been experimentally investigated. A possibility that during the animal/plant co-evolution animals could acquire the typically plant property of triterpenoid (steroidal) biosynthesis is not fully substantiated. Alternatively, predators could receive the vitamin D_1 from symbiotic, intestinal mycetomes, similarly to how it occurs in termites [7]. The most intensive cell divisions and tissue proliferation occurs during the embryonic period and in newborn animals. Provided that the conclusions on interdependence between tissue proliferation and vitamin D_1 are correct, then the most urgent need for the vitamin will be for pregnant females and newborn animals. It can thus be assumed that the vitamin D_1 should be stored in the blood of pregnant females. Moreover, it should be transferred into the milk for supporting enormous tissue proliferation and immunity in the newborn animals [5,30-34].

The first pharmacological preparation based on vitamin D_1 (20-hydroxyecdysone) was registered in the former Soviet Union under the name ECDISTEN in 1980 [3,17,24,39,51,52]. More recently, they registered another preparation named SERPISTEN, as an extract of the plant *Serratula coronata*. The preparations were advertised to produce a number of beneficial health effects, like anabolic growth effects, tonic effects, adaptogenic, neurasthenic, antifatigue, anti-inflammation and similar improvements of health conditions. There probably exist more information on ECD in Russian literature, which still have not been fully acknowledged. The frequently used and well acknowledged pharmacological preparations based on ECD depends on food additives to sport athletes for increasing their muscular performance [53]. There exist demands to impose a doping control for the presence of ECD in urine of sportsmen or racing horses [57]. These demands are conditioned by the 60-year-old categorical statements of recent phytochemists [58], that the biological status of ECD depends on the insect moulting hormone. True doping agents are anabolic, androgenic steroid hormones with oxidized steroidal side chain, which are produced in animal, never in food plants. The anabolic effects of androgenic hormones (testosterone) on the muscle systems is physiologically different from the anabolic effects of vitamin $D_{1.}$ Androgens cause increased mass of muscle tissue, while vitamin D_1 stimulates synthesis of muscular metabolic fuel, glycogen and its deposition in the muscle fibers.

There exist extensive literature data on the effects of 20-hydrocxecdyysone (vitamin $D_{1)}$ in domestic animals, especially pigs, sheep and cattle [51]. In all cases the anabolic effects characterized by increased meat and milk production, known since 1980 [17,23] have been largely confirmed. For several decades, investigations on vitamin D_1 (ECD) were mainly concerned with the location of hydroxyl groups in different species of plants [7,9,10,24-27]. The authors still adhere to the concept of insect moulting hormones. This is true also for more recent chemical publications [24-27,39,52], which are now reporting also on the anabolic, antirachitic, anticancerogenic or anti-Alzheimer´s disease effects of ECD [35,39,51,52].

The principle of carcinogenesis

For many years I studied the effects of insect juvenile hormone [42] and ECD on growth and mitotic cell divisions in insects [7,13-19,45-49] and plants [12]. It appeared that a successful realization of the mitotic divisions required the presence of 20-hydroixyecdysione (vitamin D_1) [5], not only in animals, but also in plants [12]. It is well known that vitamin D_1 occurs in hundreds of species of plants [26,39,52]. The vitamin represents a rather complex triterpenoid structure. It is produced during the vegetation season and during the winter period it is stored in seeds and roots as a resource for future tissue proliferation during the next period of growth. I trust that this unusual compound is present in all species of multicellular plants. The question appears whether it can be also produced in animals. Herbivore animals can obtain the vitamin from their diet. According to some old reports [21], insects do not synthesize triterpenoids (sterols) *de novo* and take all of it from plants. The great selective advantage of this evolutionary adaptation would be that the biosynthetic cytoplasmatic organelles of animal cells could avoid having to make the whole complex of the triterpenoid cyclisation, that is functional in plants. My experience with the regeneration of excised epidermal cells [5] revealed specific rules in maintaining tissue integrity among living cells [5,30-34]. The comparative physiology of cell division in insects shows similar rules to those in humans [56]. Thus, according to the common rules in comparative physiology, the basic facts found on vitamin D_1 in insects [5] could be also applied to humans. These comparisons led me to propose a theory explaining the principle of malignant growth [34]. The theory is summarized below in the following points:

1. Animal tissues made by living cells are grouped into physiologically integrated categories [5].
2. Individual cells of each category recognize each other by mechanical, chemical, electrical or other mutual contacts.
3. When a cell dies or a group of cells has been damaged by injury, the marginal cells which lost integrity with other living cells start to divide to restore the tissue integrity.
4. We found that the dividing cells need vitamin D_1 for the construction of cytoplasmic membranes in their daughter cells [30,33].
5. After regeneration of the wound and restoration of tissue integrity, the divisions of cells become arrested [5].

6. In absence of vitamin D_1, the dividing cells produce aberrant syncytia of cells with defective cytoplasmic membranes, which are not able to issue the signal for restored tissue integrity and arrested nuclear divisions [5,30,31].
7. The nuclei of the aberrant syncytia continue to divide, giving rise to special polynucleated metaplasia that are characteristic for the malignant tumors.
8. Application of 20-hydroxyecdysone (vitamin $D_{1)}$, 100 mg twice in a week, prevented the formation of malignant tumor metaplasia after the first chemotherapy [30].
9. It may be concluded that the polynucleated, malignant tumor metaplasia are produced under conditions when the regenerating cells must divide in the absence of vitamin D_1.

The prolonged studies in plants, insects and birds [7,12,15,18,22,50] revealed the existence of a homeostatic mechanism (preventing excess and deficiency) of vitamin D_1 in animal blood. The application of vitamin D_1 in the diet of Japanese quails was directly proportional to its content in the blood [22,50]. In the human body, excessive dosages of vitamin D_1 were rapidly excreted in urine [25]. It occurs to me that vitamin D_1 is required in all living organisms for cell divisions and tissue proliferation (spring sprouts of plants, embryonic and newborn animals). Human babies show extensive somatic growth dependent on cell divisions. They receive all nutrients and the “antirachitic” vitamin D in milk. I am reasonably thinking that milk should also contain vitamin D_1. Immunogenic properties of vitamin D_1 [17] suggest that it may be responsible for the increased immunity of newborn babies. One may wonder where do the milking mothers get the vitamin D_1 from, if it would for some reason not be present in their food? There is a possibility that the vitamin is obtained from intestinal symbiotic flora. This statement can be supported by extensive symbiotic intestinal vegetative flora of termites, who are dwelling on a vitamin deficient diet (pure cellulose). Finally, I trust that after we obtain more data about the pharmacological effects of vitamin $D_{1,}$ certain hitherto incurable diseases may also be medicated by the vitamin treatment. For example, nerve cells are long living. They do not divide. They eventually outlive the structural parts of the neurons and potentially could also be repaired using vitamin $D_{1.}$

After completion of this manuscript, I found two outstanding recent reviews on vitamin D [59,60]. The conclusions based on 100-years-old story of vitamin D are considerably different from the recent story of vitamin D_1 [5,31]. For instance, the provitamins D_2 and D_3 are seco-sterol derivatives of 7- dehydrocholesterol, purely lipid soluble, produced in animal kidney and liver, acting through the hormonal nuclear receptors, whose biological activity depends on UV irradiation of animal skin. Alternatively, I show rhar vitamin D_1 is also derivative of 7-dehydrocholesterol, with partly lipid and partly water solubility, produced like all other vitamins in plants, it is biologically active without UV-light and without the need of special nuclear receptors. The vitamin is essential for successful growth (mitotic divisions) and regeneration in all multicellular organisms on the planet. Unfortunately, due to advanced age I have no power to initiate detailed discussions, which are reserved to a younger generation of scientists.

Without respect to strict scientific regulations, I am tempted to state that since the use of vitamin $D_{1,}$ 60 years ago, I did not suffer from any viral disease. Moreover, stimulated by my earlier recommendations [30], several dozen young women diagnosed for breast cancer are living healthy lives 20 years or more, without pernicious metaplasia. They saved their life by using once a week a shot of vodka extract prepared from the roots of *Leuzea carthamoides*. I trust that the neglected vitamin D_1 will be abundant in seeds, buds and toots of many common plants.

Bibliography

1. Windaus AF and Bock F. “Über das provitamin aus dem Sterin der Schweineschwarte”. *Hoppe-Seyler´s Zeitschrift für physiologische Chemie* 245 (1937): 168-170.
2. Wolf G. “The discovery of Vitamin D: The contribution of Adolf Windaus”. *The Journal of Nutrition* 134.6 (2004): 1299-1302.

3. Windaus AF., *et al*. "Über das komplizierte Vitamin D1". *Analytical Chemistry* 489 (1931): 262-269.
4. Karlson P. "Vitamin D1 and D2". *Trends in Biochemical Sciences* 6 (1981): 29-30.
5. Sláma K. "Vitamin D1 versus ecdysteroids: Growth effects on cell regeneration and malignant growth in insects are similar to those in humans". *European Journal of Entomology* 116 (2019): 16-32.
6. Karlson P. "Ecdyson, das Häutungshormon der Insekten". *Naturwissenschaften* 53 (1965): 445-453.
7. Sláma K., *et al*. "Insect Hormones and Bioanalogues". Springer, Wien. New York. 477 (1974).
8. Nakanishi K., *et al*. "The structure of Ponasterone A, an insect molting hormone from the leaves of Podocarpus nakai Hay". *Chemical Communications* 215.24 (1966): 915-917.
9. Lafont R., *et al*. "Ecdysteroid chemistry and biochemistry". Insect Endocrinology. Elsevier B.V., Amsterdam (2011): 106-177.
10. Kumpun S., *et al*. "Ecdysteroids from *Chenopodium quinoa* Wild., an ancient Andean crop of high nutritional value". *Food Chemistry* 125.4 (2011): 1123-1234.
11. Jizba J., *et al*. "Isolation of ecdysterone (crustecdysone) from *Polypodium vulgare* L. rhizomes". *Tetrahedron Letters* 8.18 (1967): 1689-1691.
12. Macháčková I., *et al*. "Comparison between the effects of 20-hydroxyecdysone and phytohormones on growth and development in plants". *European Journal of Entomology* 92 (1995): 309-316.
13. Sláma K. "Herbivores, their interaction with secondary plant metabolites". Academic Press (1979): 673-700.
14. Sláma K. "An alternative look at insect hormones". *Life: The Excitement of Biology* 3.3 (2015): 188-204.
15. Sláma K and Zhylitskaya A. "Comprehensive physiology and toxicology of ecdysogens: he metabolically activated porphyrin ecdysteroid complexes in insects". *Comparative Biochemistry and Physiology, Toxicology and Pharmacology Part C* 181-182 (2016): 56-67.
16. Sláma K. "Ecdysteroids-insect hormones, plant defensive factors or human medicine?" *Phytoparasitica* 21 (1993): 3-8.
17. Sláma K and Lafont R. "Insect hormones-ecdysteroids: Their presence and actions in vertebrates". *European Journal of Entomology* 92 (1995): 355-378.
18. Sláma K. "The prothoracic gland revisited". *Annals of the Entomological Society of America* 91.2 (1998): 168-174.
19. Sláma K. "Illusive functions of the prothoracic gland in Galleria". *Acta Entomologica Bohemoslovaca* 80 (1983): 161-170.
20. Christakos S., *et al*. "Vitamin D: Metabolism, molecular mechanism of action, and pleiotropic effects". *Physiological Reviews* 96.1 (2016): 365-400.
21. Svoboda JA and Thompson MJ. "Steroids". *Comprehensive Insect Physiology, Biochemistry and Pharmacology* 10 (1985): 137-175.
22. Sláma K., *et al*. "Insect hormones in vertebrates; anabolic effects of 20-0hydroxyecdysone in Japanese quail". *Experientia* 52 (1995): 702-706.
23. Abubakirov NK. "Tonic preparation containing the phytoecdysone (ecdisten)". USSR Patent 1312774.06041 (1980).
24. Dinan L., *et al*. "Dietary Phytoecdysteroids". Springer Nature Singapore Pte Ltd. Xiao J., *et al*. (eds.), Handbook of Dietary Phytochemicals (2020).

25. Ambrosio G., *et al*. "Urinary elimination of ecdysterone and its metabolites following a single-dose administration in humans". *Metabolites* 11.6 (2021): 366.

26. Yamshi A., *et al*. "Phytoecdysteroids: Distribution, structural diversity, biosynthesis, activity, and crosstalk with phytohormones". *International Journal of Molecular Sciences* 23.15 (2022): 8664.

27. Głazowska J., *et al*. "Chromatographic separation, 000 determination and identification of ecdysteroids: Focus on Maral root (*Rhaponticum arthamoides, Leuzea carthamoides*)". *Journal of Separation Science* 41.23 (2018): 4304-4314.

28. Sláma K., *et al*. "Hormonal activity of ecdysteroids from certain asiatic plants". *Insect Biochemistry and Molecular Biology* 23.1 (1993): 181-185.

29. Vokáč K., *et al*. "Ecdysteroid constituents of the mushroom *Tapinella pannoides*". *Phytochemistry* 49 (1998): 2009-2114.

30. Sláma K. "Pharmacological preparation, its use and a method for determination of the risk of malignant tumors". Czech patent application PV 2018.719 (2018).

31. Slama K. "A new look at the malignant growth in regenerating animal tissue and cells". *Novel Approaches in Drug Designing and Development* 5.1 (2019).

32. Sláma K. "The neglected vitamin D1 (Polyhydroxylated 6-Keto,7-Dehydrocholesterol, Ecdysteroid): Effects on growth and regeneration in animal tissue and cells". *EC Pharmacology and Toxicology* 7 (2019): 678-690.

33. Sláma K. "Approaching a time we can prevent pernicious malignant tumors?" *EC Pharmacology and Toxicology* 8.3 (2020): 01-09.

34. Slama K. "A theory of malignant tumors induced by deficiency of vitamin D1". *EC Pharmacology and Toxicology* 10.3 (2022): 59-68.

35. Slána K. "Are ecdysteroids insect hormones?" Atlas of Science (2016).

36. Koolman J. "Ecdysone I. From Chemistry to Node of Action". 0-259, II, Biology, 260-488. Georg Thieme Verlag, Stuttgart, New York (1989).

37. Williams CM. "Physiology of insect diapause. IV. The brain and prothoracic glands as an endocrine system in the Cecropia silkworm". *Biological Bulletin* 103 (1952): 120-138.

38. Williams CM and Sláma K. "The juvenile hormone V. The sensitivity of the bug, *Pyrrhocoris apterus*, to a hormonally active factor in American paper-pulp". *Biological Bulletin* 130.2 (1966): 235-246.

39. Dinan L., *et al*. "20-Hydroxyecdysone, from plant extracts to clinical use: Therapeutic potential for the treatment of neuromuscular, cardio-metabolic and respiratory diseases". *Biomedicines* 9.5 (2021): 492.

40. Zelený J., *et al*. "Hormonally mediated inset-plant relationships: Arthropod populations associated with ecdysteroid-containing plant, *Leuzea carthamoides* (Asteraceae)". *European Journal of Entomology* 94 (1997): 83-198.

41. Stránský K., *et al*. "Lipid composition of the seeds of an ecdysteroid-containing plant, *Leuzea carthamoides* (Willd.) DC (Asteraceae)". *Russian Journal of Plant Physiology* 45.3 (1998): 390-396.

42. Sláma K. "Insect hormones: more than 50-years after the discovery of insect juvenile hormone analogues (JHA, juvenoids)". *Terrestrial Arthropod Reviews* 6 (2013): 257-333.

43. Sláma K. "Hormonal status of RH-5849 and RH-5992 synthetic ecdysone agonists (ecdysoids) examined on several standard bioassays for ecdysteroids". *European Journal of Entomology* 92 (1995): 317-323.

44. Farkaš R and Sláma K. "Effect of bisacylhydrazine ecdysteroid mimics (RH-5849 and RH-5992) on chromosomal puffing, imaginal disc proliferation and pupariation in larvae of *Drosophila melanogaster*". *Insect Biochemistry and Molecular Biology* 29.11 (1999): 1015-1102.

45. Sláma K and Weyda F. "The all-or-none rule in morphogenetic action of juvenile hormone on insect epidermal cells". *Proceedings of the Royal Society of London. Series B, Biological Sciences* 264.1387 (1997): 1463-1470.

46. Sláma K. "Correlation between metabolic depression and ecdysteroid peak during embryogenesis of the desert locust, *Schistocerca greagaria* (Orthoptera: Acrididae)". *European Journal of Entomology* 97.2 (2000): 141-148.

47. Sláma K. "Homeostatic function of ecdysteroids in ecdysis and oviposition". *Acta Entomologica Bohemoslovaca* 77 (1980): 145-168.

48. Delbecque JP and Sláma K. "Ecdysteroid titres during autonomous metamorphosis in a Dermestid beetle". *Zeitschrift für Naturforschung C* 35C.11-12 (1980): 1066-1080.

49. Slama K. "Inverse relationships between ecfysterpid titres and total body metabolism in insects". *Zeitschrift für Naturforschung C* 37c (1992): 839-844.

50. Koudela K., *et al*. "Stimulation of growth and development in Japanese quails after oral administration of ecdysteroid-containing diet". *European Journal of Entomology* 92 (1995): 349-354.

51. Dinan L and Lafont R. "Effects and applications of arthropod steroid hormones (ecdysteroids) in mammals". *Journal of Endocrinology* 191.1 (2006): 1-8.

52. Dinan L., *et al*. "Phytoecdysteroids: diversity, biosynthesis and distribution". In Smagghe G. (ed.): Ecdysone: Structures and functions. Springer Science + Business Media. B.V. Dordrecht. London (2009): 3-44.

53. Syeov VN. "On the mechanism of anabolic action of Phytoecdysteroids". *Nauch Dokl Vyssh Shk Biol Nauk* 9 (2009): 37-39.

54. Tomaschko K and Bückman D. "Excessive abundance and dynamics of unusual ecdysteroids in *Pynogonum litorale* Ström (Arthropoda, Pantopoda)". *General and Comparative Endocrinology* 90.3 (1993): 296-305.

55. Stopka P., *et al*. "Effect of insect hormone, 20-hydroxyecdysone on growth and reproduction in mice". *Acta Societatis Zoologicae Bohemicae* 63 (1999): 367-378.

56. Sláma K and Santiago-Blay JK. "Terrestrial insects with tracheae breath by actively regulating ventilatory movements: physiological similarities to humans". *The Excitement of Biology* 5.1 (2016): 4-70.

57. Karatt TK., *et al*. "An extensive screening method for the identification and quantitation of ecdysteroids in equine urine and plasma using liquid chromatography coupled with mass spectrometry". *Rapid Communications in Mass Spectrometry* 37.18 (2023): e9611.

58. Lafont R., *et al*. "Ecdysteroids". Encyclopedia. 2021, 1, 1267-1302 (202).

59. Jones G. "100 years of vitamin D: Historical aspects of vitamin D". *Endocrine Connections* 11.4 (2022): e210594.

60. Bikle DD. "Vitamin D: Production, Metabolism and Mechanisms of Action". Endotext (2021).

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